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corcl*

manufactured and sold by Funakoshi Co., Ltd., Japan) were set in a 96-well microplate chamber (catalog number: FE-2292-96, manufactured and sold by Funakoshi Co., Ltd., Japan), such that the microplate chamber is separated into a lower compartment and an upper compartment through the frame filter, wherein the microplate is fittedly accommodated in the lower compartment. The frame filter had been treated with 15 µg/ml of fibronectin (manufactured and sold under the bland of SIGMATM, U.S.A.) in PBS before being set in the microplate chamber.--

R E M A R K S

The above amendments are made in order to place the application in better grammatical format and to correct typographical errors.

Attached hereto is a marked-up version of the changes made to the specification by the current preliminary amendment. The attached page is captioned "**Version with**

markings to show changes made."

Respectfully submitted,

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By



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"Version with markings to show changes made."

brane receptor protein.

Likewise, if there can be obtained substances
which ~~respectively~~^{individually} act specifically on different types
of seven-pass transmembrane receptors (for example, the
5 chemokine receptors), such substances may possibly lead
to development of novel pharmaceuticals which each se-
lectively suppress a specific inflammatory reaction and
the like.

An explanation is made below taking chemokines as
10 an example. The chemokines and the chemokine receptors
regulate the chemotaxis of each of various different
leukocytes. Therefore, it is considered that a par-
ticular leukocyte expresses a particular chemokine re-
ceptor. Actually, there are reports that CCR5 is ex-
15 pressed by Th1 cells and CCR4 is expressed by Th2 cells
(Loetscher, P. et al., NATURE, 391, 344-345, 1998; and
Bonecchi, R. et al., J. Exp. Med., 187, 129-134, 1998),
and the chemokine receptors are considered to partici-
pate in the selection of specific cellular and humoral
20 immune responses following an antigen non-specific in-
flammation. In addition, chemokines, such as CXC and
CC chemokines, which act mainly on neutrophils and
monocytes are called "inflammatory chemokines" because
these chemokines play an important role in acute or
25 chronic inflammatory reactions. The detection of in-

the mRNA encoding C5L2 protein did not decrease even when the blood was stored for 24 hours after the collection of the blood.

As apparent from the above, although the granulocytes which are obtained from the peripheral blood of healthy donors immediately after the collection of the blood exhibits a high ratio of the expression of C5L2 gene, the ratio of the C5L2 gene expression decreases rapidly during the storage of the collected blood.

Such a phenomenon is not observed in the case of the chemokine receptors CCR4 and CCR5 and, thus, the present inventors have found that this phenomenon is specific to the receptor C5L2 of the present invention. This phenomenon has for the first time been found by the present inventors.

The regulatory mechanism of C5L2 expression in granulocytes is considered to be as follows. The leukocytes in human peripheral blood are composed of 25 to 33 % of lymphocytes, 3 to 7 % of monocytes, 55 to 60 % of neutrophils, 1 to 3 % of eosinophils and 0 to 0.7 % of basophils (see "Seikagaku Jiten (Dictionary of Biochemistry)" published by TOKYO KAGAKU DOZIN CO., LTD., Japan). As apparent from the above-mentioned composition of the leukocytes in human peripheral blood, neutrophils account for 90 % or more of the granulocyte

fraction (composed of neutrophils, eosinophils and basophils). Neutrophils participate in the non-specific immune system and they exclude pathogens (mainly bacteria) from the living body by performing various functions (such as adhesion, chemotaxis, phagocytosis and bactericide). A mature neutrophil stays in human peripheral blood for 10 to 16 hours and its life is 2 to 3 days. Mature neutrophils are either those neutrophils circulating in the blood or those neutrophils which are in a form adhering to vascular endothelium (i.e., vascular endothelial cells covering the inside wall of a blood vessel). Under normal conditions, the number of mature neutrophils circulating in the blood and the number of mature neutrophils in a form adhering to vascular endothelium are approximately the same.

The mature neutrophils which have been in a form adhering to vascular endothelium will then emigrate into tissues, and such neutrophils are lost as they go out of the tissues and into a space outside the tissues (such as the oral cavity, a gastrointestinal lumen and the internal space of a pulmonary alveolus) or, in the case of some tissues (such as liver, spleen, hypodermal tissue and the like), the neutrophils undergo apoptosis and are then ^{phagocytosed} phagocytosed by macrophages. From these facts, it is considered that the average life of mature

neutrophils which emigrate into tissues is 1 to 4 days. A mature neutrophil which has emigrated into a tissue does not return to the blood. With respect to the mature neutrophils which migrate to a site of inflammation, the apoptosis of these neutrophils is regulated so that the life of these neutrophils is prolonged. On the other hand, the life of the mature neutrophils which have ~~phagocytosed~~ ^{phagocytosed} bacteria is shortened. When a mature neutrophil undergoes apoptosis, its various functions (such as chemotaxis, phagocytosis, morphological change, adhesion, degranulation and production of active oxygen) are lowered (Haslett, C. et al., Chest, 99 (Suppl. 3): 6S, 1991; and Whyte, M.K. et al., J. Immunol., 150, 5124-5134, 1993). Such an onset of neutrophil apoptosis is considered to be caused by a mechanism working for preventing the occurrence of disorders caused by a prolonged activation of neutrophils, which is harmful for the living body.

It is considered that the receptor C5L2 of the present invention is usually expressed on the cells, and that when a disorder (such as an infection or an inflammation) occurs in the living body, the cells expressing C5L2 receptor emigrate into the site of the disorder and act on the disorder. That is, the receptor C5L2 is expressed on the cells which are capable of

Example 7

Screening of a ligand

CHO cells (ATCC number: CCL-61, available from Dainippon Pharmaceutical Co., Ltd., Japan) were transformed with pcDNAC5L2 constructed in Example 4. The CHO cells were cultured at 37 °C in an atmosphere containing 5 % of carbon dioxide using F-12 nutrient mixture (Ham's F-12, catalog number: 11765-047, manufactured and sold under the brand of GIBCO BRL™, U.S.A.) containing 10 % FBS (catalog number: 10099-141, manufactured and sold under the brand of GIBCO BRL™, U.S.A.) and 1 % (vol/vol) of Penicillin-Streptomycin. The transformation was conducted by the calcium phosphate co-precipitation method using Calcium Phosphate Transfection Kit in accordance with the protocol attached thereto. DNA was used in an amount of 5 µg per plate (diameter: 35 mm).

After the transformation, the cells were transferred to a medium containing 400 µg/ml Geneticin (catalog number: 11811-023, manufactured and sold under the brand of GIBCO BRL™, U.S.A.) in various cell numbers. The cells were grown for approximately two weeks and the grown cells were used in the experiment below as C5L2 protein-expressing CHO cells.

Using the above-mentioned C5L2 protein-expressing

CHO cells, the chemotaxis of the cells was observed. As a sample material containing a ligand candidate substance, an LPS (lipopolysaccharide)-administered rat serum was used, which had been prepared as follows.

5 *Salmonella minnesota* Re 595-derived LPS (manufactured and sold under the brand of SIGMATM, U.S.A.) was suspended in a physiological saline, wherein the LPS was used in an amount such that the final concentration thereof became 1 mg/ml. The resultant suspension was
10 sonicated by means of a sonicator (manufactured and sold by Branson, Japan) to thereby obtain a transparent solution. The obtained solution was diluted ten-fold with the physiological saline and 400 µl of the diluted solution was intravenously administered to a 7-week-old
15 Wistar rat (bought from NIPPON BIO-SUPP CENTER, Japan) at the tail thereof. About two hours after the administration, the rat was etherized and laparotomized, and then, blood was collected from the heart thereof. The collected blood was centrifuged at 13,000 rpm for 15
20 minutes at 4 °C by means of a microtube centrifuge. From the resultant centrifuged blood, a supernatant was separated and used as a sample material containing a ligand candidate substance.

25 A 96-well microplate (catalog number: FE-2300-02, manufactured and sold by Funakoshi Co., Ltd., Japan)

and a frame filter (pore size: 8 μm) (catalog number: FE-2340-08, manufactured and sold by Funakoshi Co., Ltd., Japan) ^{were} was set in a 96-well microplate chamber (catalog number: FE-2292-96, manufactured and sold by Funakoshi Co., Ltd., Japan), such that the microplate chamber is separated into a lower compartment and an upper compartment through the frame filter, wherein the microplate is fittedly accommodated in the lower compartment. The frame filter had been treated with 15 $\mu\text{g}/\text{ml}$ of fibronectin (manufactured and sold under the brand of SIGMATM, U.S.A.) in PBS before being set in the microplate chamber.

The sample material was diluted ten-fold with RPMI1640 medium (catalog number: 22400-071, manufactured and sold under the brand of GIBCO BRLTM, U.S.A.) containing 0.15 % BSA (bovine serum albumin), and the resultant solution was added to the lower compartment of the microplate chamber. The C5L2 protein-expressing CHO cells suspended in RPMI1640 medium containing 0.15% BSA was added to the upper compartment of the microplate chamber, and then, the microplate chamber was incubated at 37 °C for 5 hours under an atmosphere containing 5 % of carbon dioxide, to thereby contact the sample material with the C5L2 protein expressed on the cells. Subsequently, the frame filter was removed from